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⑰ Multicuvette rotor for use in a centrifugal analyzer.

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Courier Press, Leamington Spa, England.

Description

This invention relates to analytical systems, and more particularly to multicuvette rotors for use in centrifugal analyzer systems.

Centrifugal chemical analysis systems employ a cuvette rotor assembly that defines a circumferential array of spaced elongated radially extending cuvettes, each of which has two chambers for initially storing reagent materials separately and then transferring reagent material from one chamber to another for mixing and reaction, and subsequent analysis of the reaction in the analysis region by the cooperating analyzer. Cuvette rotor assemblies of this type are disclosed in Tiffany et al. U.S. Patent 4,226,531 upon the disclosure of which is based the prior art portion of claim 1, and Stein et al. U.S. Patent 4,373,812. In use of such rotors, sample to be analyzed (frequently with supplemental reagent material) is introduced through a loading port into one chamber and a second reagent material is introduced through a second loading port into the second chamber. The rotors disclosed in the above mentioned patents have twenty cuvettes that are loaded successively with automated loading equipment, small quantities of sample plus optional quantities of diluent and second reagent (volumes typically in the range of 2—100 microliters) being pipetted through loading ports into first chambers, and reagents in quantities up to 200 microliters being pipetted through loading ports into second chambers. The loaded cuvette rotor is then transferred to an analyzer for photometric, light scatter and/or fluorescence analysis. During the analysis sequence, the rotor usually is driven at a preliminary fast speed in the vicinity of 3000—5000 rpm in which the reactant in each inner chamber flows over divider structure and mixes with the reactant in the outer chamber, and then the rotor is driven at speeds in the vicinity of 500—1000 rpm during a measurement interval.

Numerous analytical tests are performed with such analyzers including, for example, glucose, creatinine, CPK, SGOT, triglyceride, and enzyme immunoassays. It has been found that there is an unacceptable tendency for reagent material to spontaneously move or "wick" along the upper surface of the cuvette resulting in premature mixing of reagents between the two chamber compartments, such mixing occurring in the case of some tests in less than one minute after loading, while the loading sequence may take five minutes or more. For example, a drop of sample or reagent material (of several microliters in size) may adhere to a cuvette sidewall and transfer from there along a capillary channel between the top of the cuvette sidewall and the cover into the chamber from which it is supposed to be excluded until the reaction is initiated by a centrifuging action. This problem is particularly significant with reagent materials that have high wettability characteristics such as reagents used in enzyme immunoassays, for example.

The present invention as defined in claim 1, and

as later described by way of example, provides for the manufacture of a rotor that includes a one-piece body member of material that is transparent at analytical wavelengths and that has a planar upper surface and that defines a circumferential array of elongated cuvette recesses, and a one-piece cover member of similarly transparent material that has a planar lower surface parallel to the planar upper surface of the body member. A continuous seal extends around each cuvette recess between the planar upper and lower surfaces to define the circumferential array of analytical cuvettes. A difficulty of the prior art is that a capillary channel is frequently formed at the junction between the cover and body members so that there is a tendency for premature mixing of the constituents to occur due to wicking movement of a constituent stored in one of the chamber regions to the other chamber region along that capillary channel at the junction. The present invention overcomes this problem by having a carefully dimensioned ridge spaced the correct distance from the side wall of the cuvette in order reliably to fill the junction between the cover and body members so that no capillary channel is left which could lead to premature mixing of constituents from different chamber regions. Thus the ridges are located such that, after welding, the portion of the ridge material which has "melted" fills the void between the top cover and the top of the cuvette sidewall and a smooth, radiused junction is formed between the two components of the rotor assembly. Hereinafter we will frequently refer to the ridges as being energy director ridges or being of energy director material since the ridges are of a sharp crested form such that upon initial contact with a cooperating member subject to ultrasonic vibration the sharp crest tends to direct the energy input such that it is that crest which initially melts and flows to the extent required to give the good barrier seal filling the junction between body and cover members. While the energy director ridge seal and barrier structures may be formed on either the body member or the cover member, or partly on each, preferably the barrier and seal structures are integral with the body member. By properly positioning the energy director ridges on the rotor body and by using two ridges along the top surface of each sidewall defining web between adjacent cuvettes, the required amount of energy director ridge material is reduced and less uncertainty in the finished (welded) rotor optical path length between cuvettes and between rotors results.

While US—A—4123173 and 4314970 disclose cuvette rotors with sealing between a cover and a cuvette containing base, neither provides for the ready and reliable manufacture of a rotor in which due to the spacing, dimensions and location of the energy director ridges between adjacent cuvettes a capillary channel free junction can reliably be obtained with the minimum application of material melting energy.

In a particular embodiment, the rotor assembly

has a diameter of about ten centimeters and an overall height of about one centimeter, the cover member is a flat circular disc that has an optical window region, an outer circumferential array of loading ports, an inner circumferential array of loading ports, and a substantially "D" shaped central opening; and the body member has a flat upper surface, an optical window region formed in its lower surface that is aligned with the optical window region of the cover member and a circumferential array of thirty-nine individual cuvette recesses. Each cuvette recess of that rotor has a length of about three centimeters, the chamber sidewalls diverge at an angle of about nine degrees from the center of the rotor, and the analytical region (defined by the pair of opposed optical windows adjacent the outer periphery of the rotor disc) has parallel side walls. That rotor embodiment includes triangular energy director ridge portions that have a base width of about 0.1 millimeter, a height of about 0.1 millimeter, and are set back about 0.1 millimeter from and extend parallel to the adjacent top edge of the cuvette side wall. The web thickness between each cuvette is small—less than 0.3 centimeter, and a pair of parallel energy director ridges are integrally formed on the planar upper surface of each such web.

In centrifugal analyzer rotors in accordance with the invention, spontaneous mixing of sample and reagent due to wicking along the junction between cover and cuvette side walls is significantly impeded without increase in the size of the rotor and with significant increase in the number of cuvettes in the rotor assembly.

Other features and advantages of the invention will be seen as the following description of a particular embodiment progresses, in conjunction with the drawings, in which:

Fig. 1 is a top plan view (with portions broken away) of a multicuvette rotor assembly in accordance with the invention;

Fig. 2 is a sectional view taken along the line 2—2 of the rotor assembly shown in Fig. 1;

Fig. 3 is an enlarged view of a portion of the body member of the rotor assembly shown in Fig. 1;

Figs. 4 and 5 are still further enlarged views of portions of the body member indicated at 4—4 and 5—5 in Fig. 3;

Fig. 6 is a sectional view taken along the line 6—6 of Fig. 4;

Fig. 7 is a sectional view taken along the line 7—7 of Fig. 4;

Fig. 8 is a sectional view taken along the line 8—8 of Fig. 5;

Fig. 9 is an enlarged sectional view taken along the line 9—9 of Fig. 1; and

Fig. 10 is a further enlarged view of the portion indicated at 10—10 in Fig. 9.

Description of particular embodiment

With reference to Figs. 1 and 2, rotor assembly 1 has a diameter of about ten centimeters and an overall height of about 1 1/4 centimeters and is

formed of an injection-molded acrylic cover member 12 and an injection-molded acrylic body-member 14 that are ultrasonically welded together, the body and cover members having appropriate transparency, chemical resistance, and optical characteristics for photometric analysis. Rotor assembly 10 defines a circumferential array of thirty-nine individual analysis cuvettes 16 (plus a reference region of similar configuration) and has circumferential flange structure 20 at its periphery in which are formed alignment recesses 22, 24, and with a continuous circumferential surface 26 below flange 20 in which is located a series of optical end windows, one for each cuvette 16. Optical window channel recess 30 (about 1/2 centimeter wide with its inner edge at about four centimeters radius) is formed in the upper surface of rotor assembly 10, and a corresponding continuous annular optical window channel recess 32 is formed in the lower surface of rotor 10, channels 30, 32 being aligned and defining cuvette analysis regions 34 therebetween.

Formed in cover member 12 (as indicated in Fig. 1) are a substantially D-shaped central opening 38, a series of three arcuate spacer ribs 40, a circumferential array of first loading ports 42, a second circumferential array of second loading ports 44 with depending tubular extensions 46, and annular recessed optical window channel 30 outwardly of ports 44 and adjacent rim 20.

The thirty-nine analysis cuvettes 16 are of the same configuration, each cuvette 16 having a length of about three centimeters between cylindrical inner wall surface 50 and planar outer wall 52 surface that has an optical surface finish of better than three microinches and which defines the inner surface of optical window 28. Each cuvette 16 has an inner chamber portion 54 (which is loaded through its port 42) and an outer chamber portion 56 (which is loaded through its port 44) that are separated by divider ramp structure 58. The surfaces 60 of each cuvette that define the side walls of chambers 54 and 56 are formed by solid webs 62 that are about one millimeter thick and diverge at an angle of nine degrees. As may be seen with reference to Figs. 1—4, the analysis region 34 of each cuvette is bounded by parallel side wall surfaces 64 (spaced about 0.5 centimeter apart) adjacent outer wall 52, and short transition wall surfaces 66 (inclined at an angle of about 30 degrees) connect diverging side wall surfaces 60 of chambers 56 (where they are spaced about 5.5 millimeters apart) and side wall surfaces 64 of analysis regions 34.

The two aligned optical window channels 30, 32 are each about 1/2 centimeter wide, with the inner edges 68 of channels 30, 32 located slightly radially inwardly of transition surfaces 66 of cuvette 16. The upper and lower surfaces 70, 72 in analysis region 34 have optical finishes of better than three microinches (as have surfaces 30, 32 and 52) and are spaced about 3/4 centimeter apart to provide an optical path 74 (Fig. 2) of about 3/4 centimeter length in each analysis region 34.

Further details of body member 14 may be seen with reference to Figs. 2—7. Member 14 has planar upper surface 76 on which is formed an interconnected array of energy director ridges 80 so that there is continuous ridge structure that extends about the perimeter of each individual cuvette 34, as indicated in Figs. 1—3. Each energy director ridge 80 extends parallel to and is set back about 0.1 millimeter from the edge of the adjacent cuvette wall sections 50, 52, 60, 64, and 66 and is of triangular cross-sectional configuration. With reference to Figs. 6—8, each ridge 80 has a base width of about 0.1 millimeter and a peak 82 that has a height of about 0.1 millimeter; and the energy director ridges 80 include inner ring section 84, outer ring section 86, and sidewall sections 88.

In sealing cover 12 to body 14, cover 12 is placed on the upper surface 76 of body 14 and then ultrasonically welded to body 14 with a horn pressure of about 60 psi and application of twenty kilohertz energy for about one second. That ultrasonic energy and pressure melts and flattens the energy director ridges 80 as indicated at 90 in Figs. 9 and 10 with ridge material flowing to the sidewall of the cuvette, and creating a peripheral seal of melted plastic material (as indicated at 90 in Figs. 9 and 10) about each cuvette 16. The flow of melted plastic material 90 along surface 76 to side wall 60 (50, 52, 64, 66) smoothly fills the gap between cover surface 70 and body surface 76 adjacent cuvette side wall portions 50, 52, 60, 64, 66. Such a gap in prior rotors has been found to induce rapid capillary "wicking" action that transfers any reagent that contacts it from one compartment 54 or 56 to the other compartment with resultant premature mixing, and the smooth joinings of surfaces 70 and 76 by the flowed, flattened melted energy director ridge structures 80 inhibit spontaneous premixing of reagent materials.

In use of this rotor embodiment, a selected sample volume in the range of 2—20 microliters (optionally with an additional volume of supplemental reagent material or diluent) is dispensed into chamber 54 and a selected reagent volume in the range of 150—200 microliters is dispensed into chamber 56 depending on the particular test involved. As indicated above, potential wicking action is inhibited by the melted structures 90 of energy director ridges 80 such that spontaneous mixing of reagents due to such capillary wicking action between the two chambers of the cuvette is essentially prevented.

After some or all of the thirty-nine cuvettes 16 of rotor 10 have been loaded, the rotor is transferred to an analyzer for incubation (if necessary), centrifugal acceleration to provide transfer of the sample (and reagent) from chamber 54 to analysis chamber 34 and mixing. The rotor is the braked and then accelerated again to about 600 rpm for photometric analysis. The barrier structures 90 of melted ridges 80 retard spontaneous 'wicking' and 'creep' type flows of reagent in either direction from one chamber to the other while not interfering with transfer of sample and

reagent from chambers 54, 56 to region 34 under centrifugal force nor with the mixing and analysis steps.

5 Claims

1. A method of making a multicuvette rotor for use in a centrifugal analyser, said method comprising providing a one-piece body member (14) of transparent material that has a planar upper surface (76) and that is provided with a circumferential array of elongated cuvette recesses, and a one-piece cover member (12) of transparent material that is fittable to the body member with a planar lower surface (70) parallel to the planar upper surface of the body member, each said elongated cuvette recess having a first chamber region (54) for receiving a first constituent and first loading ports (42) being provided in the cover member for introduction of the first constituent into the first chamber regions during use of the rotor, the cover member also being provided with second loading ports through which the second constituent is introducible into the second chamber regions, the cuvette recesses each being provided with divider structure between the first and second chamber regions, said divider structure having a crest portion spaced from the lower surface of the cover member when the cover member is fitted to the body member so that a transfer passage from the first and second chamber regions is defined between the crest portion and the lower surface of the cover member for the flow therethrough of the first constituent in the second chamber region for forming a reaction product with the second constituent during use of the rotor, an analysis region (34) being provided adjacent the radially outer wall of each cuvette recess, said reaction product being subjectable to analysis in such analysis region, one of the members having formed thereon a sharp crested ridge of the transparent material extending completely around the contour of each cuvette recess, said method also comprising applying the cover member to the body member and subjecting the cover member to ultrasonic vibration whereby the sharp crests of the ridges on the one of the members are pressed against the other member and are caused by such ultrasonic vibration to melt to provide a barrier structure that extends as a continuous seal around each cuvette recess, characterised in that each pair of adjacent cuvette recesses are separated along their entire radial lengths by two of said ridges with each ridge being spaced from the edge of the adjacent cuvette recess side wall by substantially the same distance as the height of said ridge, and in that the sharp crest of these ridges are melted and flattened by the ultrasonic heating to provide the barrier structure that extends along the top of each side wall of each cuvette recess to fill the junction between the cover and body members to that there is no capillary channel at the junction, such that, in use of the rotor, premature mixing of the constituents

due to wicking movement of a constituent stored in one of said chamber regions to the other chamber region along the junction is inhibited, each cuvette recess being completely surrounded by a ridge individual thereto immediately prior to ultrasonic heating.

2. A method according to claim 1, wherein the ridge before melting has a height of about 0.1 mm.

3. A method according to claim 1 or 2, wherein the material of said ridge is integral with the body member.

4. A method according to any preceding claim, wherein the cover member is a flat circular disc that has an optical window region, an outer circumferential array of loading ports, an inner circumferential array of loading ports, and a substantially "D" shaped central opening; and said body member has a flat upper surface, an optical window region formed in its lower surface that is aligned with the optical window region of said cover member when said cover member is fixed thereto, and a circumferential array of individual cuvette recesses corresponding with said lower loading ports.

5. A method according to any preceding claim, wherein each cuvette recess has a length of about 3 centimetres, the side walls of said chambers diverge, and the side walls of the analytical regions are parallel.

Patentansprüche

1. Verfahren zum Herstellen eines Rotors mit mehreren Küvetten für die Verwendung in einem Zentrifugalanalysator, wobei das Verfahren aufweist: Bereitstellen eines einstückigen Grundkörperteiles (14) aus einem durchsichtigen Material, welches eine ebene obere Oberfläche (76) aufweist und mit einer umlaufenden Reihe von länglichen Küvettenaussparungen versehen ist, und Bereitstellen eines einstückigen Deckelteiles (12) aus einem durchsichtigen Material, welches mit einer ebenen unteren Oberfläche (70) parallel zu der ebenen oberen Oberfläche des Grundkörperteiles auf das Grundkörperteil aufsetzbar ist, wobei jede längliche Küvettenaussparung einen ersten Kammerbereich (54) für die Aufnahme eines ersten Bestandteiles sowie erste Beschickungsöffnungen (42) hat, die in dem Deckelteil für die Einführung des ersten Bestandteiles in die ersten Kammerbereiche während des Gebrauchs des Rotors vorgesehen sind, wobei das Deckelteil auch mit zweiten Beschickungsöffnungen versehen ist, durch welche der zweite Bestandteil in zweite Kammerbereiche einführbar ist, wobei die Küvettenaussparungen jeweils mit einem Teilungsaufbau zwischen den ersten und zweiten Kammerbereichen versehen sind, wobei der Teilungsaufbau ein Kammteil bzw. einen Grat hat, der von der unteren Oberfläche des Deckelteiles beabstandet ist, wenn das Deckelteil auf das Hauptkörperteil aufgesetzt ist, so daß zwischen dem Grat und der unteren Oberfläche des Deckelteiles ein Überführungsdurchgang aus den ersten

und zweiten Kammerbereichen definiert ist für den Hindurchfluß des ersten Bestandteiles in den zweiten Kammerbereich, um ein Reaktionsprodukt mit dem zweiten Bestandteil während des Gebrauchs des Rotors zu bilden, wobei ein Analysebereich (34) neben der radial außenliegenden Wand jeder Küvettenaussparung vorgesehen ist und wobei das Reaktionsprodukt in einem solchen Analysebereich einer Analyse unterworfen werden kann, wobei an einem der Teile aus durchsichtigem Material ein scharfgratiger Steg angeformt ist, welcher sich vollständig um den Umriß jeder Küvettenaussparung erstreckt, wobei das Verfahren auch das Anbringen des Deckelteiles an dem Hauptkörperteil und das Aussetzen des Deckelteiles einer Ultraschallschwingung einschließt, wodurch der scharfe Grat der Stege an einem der beiden Teile gegen das andere Teil gedrückt und veranlaßt wird, durch die Ultraschallschwingung aufzuschmelzen, um einen Barrierenaufbau bereitzustellen, der sich als eine kontinuierliche Dichtung um jede Küvettenaussparung herum erstreckt, dadurch gekennzeichnet, daß jedes Paar von benachbarten Küvettenaussparungen entlang deren gesamter radialen Länge durch zwei derartige Stege getrennt sind, wobei jeder Steg vom Rand der benachbarten Seitenwand der Küvettenaussparung durch im wesentlichen den gleichen Abstand beabstandet ist, wie der Steg hoch ist, und daß der scharfe Grat dieser Stege durch die Ultraschallheizung aufgeschmolzen und abgeflacht wird, um zu gewährleisten, daß der Grenzaufbau, der sich entlang der Oberseite jeder Seitenwand jeder Küvettenaussparung erstreckt, den Übergang zwischen dem Deckel und dem Grundkörperteil ausfüllt, so daß es keinen Kapillarkanal an diesem Übergang gibt, derart, daß beim Gebrauch des Rotors eine Vermischung der Bestandteile aufgrund der Bewegung eines in einem der Kammerbereiche aufbewahrten Bestandteiles durch Kapillareffekte entlang des Überganges in den anderen Kammerbereich hinein verhindert wird, wobei jede Küvettenaussparung unmittelbar vor dem Ultraschallheizen vollständig von einem Steg umgeben ist, der allein zu ihr gehört.

2. Verfahren nach Anspruch 1, wobei der Steg vor dem Schmelzen eine Höhe von etwa 0,1 mm hat.

3. Verfahren nach Anspruch 1 oder 2, wobei das Material des Steges einstückig mit dem Grundkörperteil ist.

4. Verfahren nach einem der vorstehenden Ansprüche, wobei das Deckelteil eine flache kreisförmige Scheibe ist, welche einen Bereich mit einem optischen Fenster hat, eine äußere umlaufende Reihe von Beschickungsöffnungen, eine innere umlaufende Reihe von Beschickungsöffnungen und eine im wesentlichen "D"-förmige zentrale Öffnung, und wobei das Grundkörperteil eine flache obere Oberfläche hat, einen optischen Fensterbereich hat, welcher in seiner unteren Oberfläche ausgebildet ist, und welcher mit dem optischen Fensterbereich des Deckelteiles ausgerichtet ist, wenn das Deckelteil daran befestigt ist,

und eine umlaufende Reihe von einzelnen Küvettenaussparungen hat, welche den unteren Beschickungsöffnungen entsprechen.

5. Verfahren nach einem der vorstehenden Ansprüche, wobei jede Küvettenaussparung eine Länge von etwa 3 cm hat, die Seitenwände der Kammern divergieren und die Seitenwände der Analysebereiche parallel sind.

Revendications

1. Procédé destiné à réaliser un rotor à cuvettes multiples en usage dans un analyseur centrifuge, comprenant les étapes consistant à fournir un élément de corps d'une pièce (14) en matière transparente qui comporte une surface supérieure plane (76) qui est munie d'une série circonférentielle d'évidements de cuvettes allongées, et un élément de couvercle d'une pièce (12) en matière transparente qui peut s'adapter sur l'élément de corps avec une surface inférieure plane (70) parallèle à la surface supérieure plane de l'élément de corps, chaque évidement de cuvette allongée comportant une première région de chambres (54) destinée à recevoir un premier constituant et des premiers orifices de chargement (42) étant prévus dans l'élément de couvercle pour l'introduction du premier constituant dans les premières régions de chambres pendant l'utilisation du rotor, l'élément de couvercle étant également muni de seconds orifices de chargement à travers lesquels le second constituant peut être introduit dans les secondes régions de chambres, chaque évidement de cuvette étant muni d'une structure de séparation entre les premières et secondes régions de chambres, cette structure de séparation présentant une portion d'arête espacée de la surface inférieure de l'élément de couverture lorsque l'élément de couverture est fixée sur l'élément de corps de sorte qu'un passage de transfert depuis les premières et secondes régions de chambres est défini entre la portion d'arête et la surface inférieure de l'élément de couvercle pour l'écoulement à travers celui-ci du premier constituant dans la seconde région de chambres pour former un produit réactionnel avec le second constituant pendant l'utilisation du rotor, une région d'analyse (24) étant prévue de façon contiguë à la paroi radialement extérieure de chaque évidement de cuvette, ce produit réactionnel pouvant être soumis à analyse dans cette région d'analyse, l'un des éléments comportant formée sur celui-ci une nervure à arête vive en matière transparente s'étendant complètement sur le contour de chaque évidement de cuvettes, ce procédé de fabrication comprenant également l'application de l'élément de couvercle sur l'élément de corps et la soumis-

sion de l'élément de couvercle à des vibrations par ultrasons, moyennant quoi les arêtes vives des nervures sur l'un des éléments sont comprimées contre l'autre élément et sont mises en fusion par cette vibration ultrasonore pour fournir une structure de barrière qui s'étend sous forme de joint continu autour de chaque évidement de cuvette, caractérisé en ce que chaque paire d'évidements de cuvettes contiguës est séparée le long de sa longueur radiale complète par deux des nervures avec chaque nervure espacée depuis le bord de la paroi latérale de l'évidement de cuvettes contiguës de sensiblement la même distance que la hauteur de cette nervure, et en ce que l'arête vive de ces nervures est mise en fusion et aplatie par le chauffage par ultrasons pour fournir une structure de barrières qui s'étend le long du sommet de chaque paroi latérale de chaque évidement de cuvettes pour remplir la jonction entre les éléments de cuvettes et de corps de sorte qu'aucun canal capillaire n'est formé au niveau de cette liaison, et que de cette manière, dans l'utilisation du rotor, un mélange prématuré des constituants par suite du mouvement d'absorption d'un constituant stocké dans l'une des régions de chambres vers l'autre région de chambre le long de cette liaison est inhibé, chaque évidement de cuvette étant complètement entouré d'une nervure individuelle immédiatement avant le chauffage par ultrasons.

2. Procédé selon la revendication 1 dans lequel avant la fusion la nervure présente une hauteur d'environ 0,1 mm.

3. Procédé selon la revendication 1 ou 2, dans lequel le matériau de la nervure est solidaire de l'élément de corps.

4. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'élément de couvercle est une disque circulaire plat qui présente une région de fenêtres optiques, une série circonférentielle extérieure d'orifices de chargement, une série circonférentielle intérieure d'orifices de chargement, et une ouverture centrale sensiblement en forme de "D"; et cet élément de corps comporte une surface supérieure plate, une région de fenêtres optiques formée dans sa surface inférieure qui est alignée sur la région de fenêtres optiques de cet élément de couvercle lorsque l'élément de couvercle est fixé sur celui-ci, et une série circonférentielle d'évidements de cuvettes individuelles correspondant aux orifices de chargement inférieurs.

5. Procédé selon l'une quelconque des revendications précédentes, dans lequel chaque évidement de cuvette présente une longueur d'environ 3 cm, les parois latérales des chambres divergent, et les parois latérales des régions d'analyse sont parallèles.

FIG 1

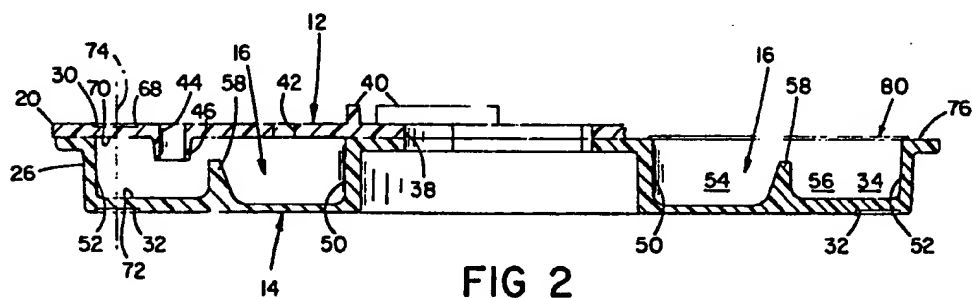
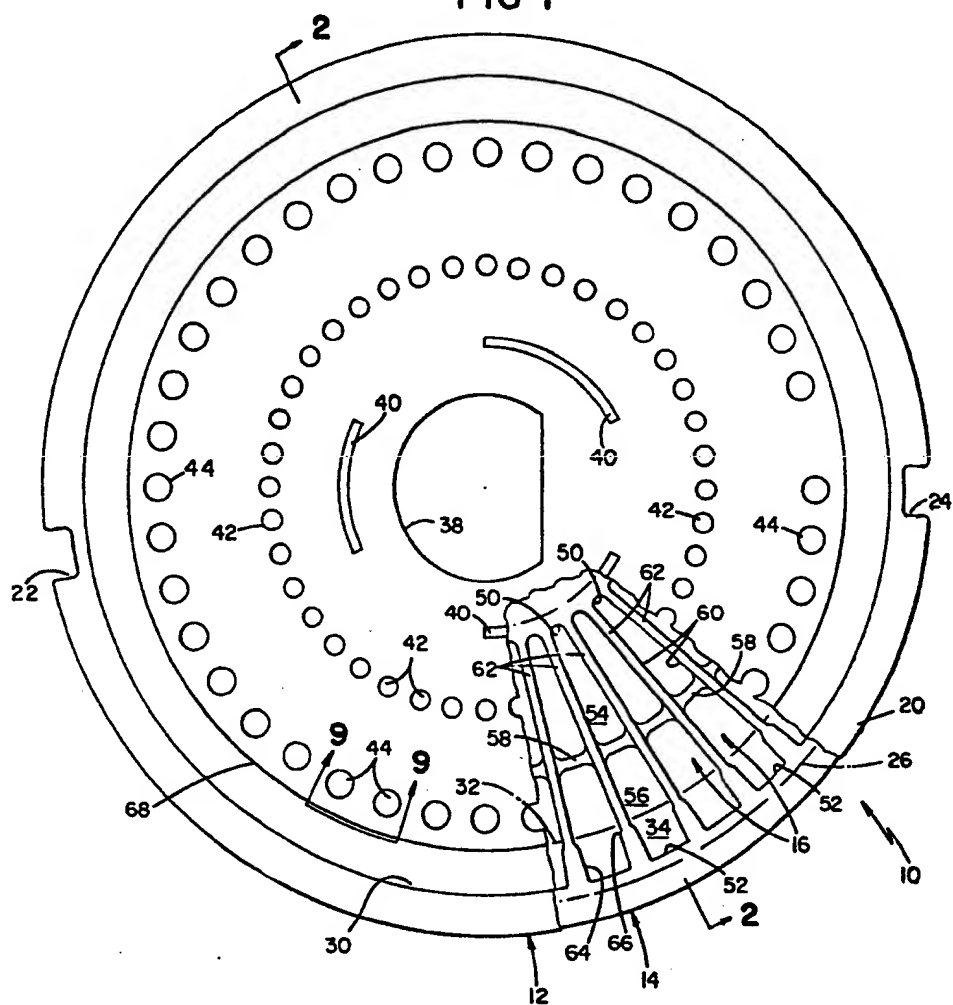


FIG 2

FIG 3

